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Sleep enhances memory consolidation in children

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Abstract

Sleep is an active state that plays an important role in the consolidation of memory. It has been found to enhance explicit memories in both adults and children. However, in contrast to adults, children do not always show a sleep-related improvement in implicit learning. The majority of research on sleep-dependent memory consolidation focuses on adults; hence the current study examined sleep-related effects on two tasks in children. Thirty-three typically developing children aged 6 to 12 years took part in the study. Actigraphy was used to monitor sleep. Sleep-dependent memory consolidation was assessed using a novel non-word learning task and the Tower of Hanoi cognitive puzzle, which involves discovering an underlying rule to aid completion. Children were trained on the two tasks and retested following approximately equal retention intervals of both wake and sleep. After sleep, children showed significant improvements in performance of 14% on the non-word learning task and 25% on the Tower of Hanoi task, but no significant change in score following the wake retention interval. Improved performance on the Tower of Hanoi may have been due to children consolidating explicit aspects of the task, for example rule-learning or memory of previous sequences; thus we propose that sleep is necessary for consolidation of explicit memory in children. Sleep quality and duration were not related to children's task performance. If such experimental sleep-related learning enhancement is generalizable to everyday life, then it is clear that sleep plays a vital role in children's educational attainment.

Abbreviations: REM rapid eye movement, Non-REM non-rapid eye movement, SWS slow wave sleep

Keywords: Sleep, Sleep-dependent memory consolidation, Paediatric, Memory

Sleep is an active physiological state characterized by a distinct cyclical pattern of activation and deactivation of cortical and subcortical structures. Neural activity travels through three progressively deeper stages of non-rapid eye movement (non-REM) sleep before entering rapid eye movement (REM) sleep. This cycle repeats throughout the night. Relative to adults, children have shorter sleep cycles and longer total sleep time of around ten hours per night at school age. REM dominates in infancy, whilst stage III of non-REM, also known as slow wave sleep (SWS), occupies a greater proportion of total sleep time throughout childhood, but the proportion gradually decreases, whilst stage II increases accordingly (Kahn et al. 1996; Ohayon et al. 2004).

Sleep is necessary for optimum physiological and psychological functioning, including the ability to form and retrieve certain types of memories. Sleep-dependent memory consolidation is the phenomenon whereby memory traces are preferentially consolidated during sleep as opposed to wake, leading to improved performance following a retention interval of sleep, even without further physical practice (Wilhelm et al. 2008; Walker et al. 2002). Broadly, two types of memory can be defined: explicit or declarative memory ('knowing that') is knowledge of information that can be easily put into words and tested by direct questioning, whilst implicit or procedural memory ('knowing how') is knowledge of procedures or skills, is difficult to verbalize and is measured by gains in speed and/or accuracy on a skill-based task. The terms explicit/implicit instead of declarative/procedural are used in this paper since, in adults, the Tower of Hanoi task, used in the current study, relies on implicit learning to discover a hidden rule, rather than procedural skills per se (Smith et al., 2004). Although learning rarely relies solely on one function, the explicit/implicit or declarative/procedural dichotomy is commonly used to define tasks for testing purposes (e.g., Plihal and Born, 1997; Wilhelm et al., 2008).

Research in adults suggests that consolidation of explicit memories is particularly dependent upon SWS whilst implicit memories benefit from REM sleep (Maquet et al. 2000; Gais and Born 2004; Plihal and Born 1997, though see also Rasch et al., 2008). Stage II also appears to play a part in reinforcing both types of memory (Fogel and Smith 2006; Walker et al. 2002). This process may be coordinated by the hippocampus, an area of the brain involved in memory encoding, consolidation and retrieval. The hippocampus reactivates new memories during sleep, thus enabling the strengthening of neural pathways and transfer of information to long-term storage in cortical regions (Maquet et al. 2000; Bergmann et al. 2012).

Child development is characterized by rapid learning of factual information, language, motor skills and behaviours which are likely to be enhanced by sleep. Increased REM in infancy and SWS throughout childhood could reflect this extensive learning, promoting neural plasticity involved in off-line consolidation (Wilhelm et al. 2012b).

Recent research on sleep-dependent memory consolidation has brought to light key differences between children and adults (e.g., Wilhelm et al. 2008). For both adults and children explicit memories have been found to be preferentially enhanced by sleep compared to wake. Studies using word pair tasks, where the participant must recall the paired word when prompted with the first word of the pair, show that children, adolescents and adults remember more word pairs after a retention interval of sleep than following an equivalent period of wake, regardless of whether initial training takes place in the morning or evening (e.g., Plihal and Born 1997; Potkin and Bunney 2012; Wilhelm et al. 2008) Sleep has also been implicated in the consolidation of novel non-words in both adults (Davis et al. 2009) and children (Henderson et al. 2012). The findings suggest an active role of sleep in off-line explicit memory consolidation, and consistency throughout development.

For implicit knowledge, evidence for sleep-dependent memory consolidation in adults is fairly robust and reliably finds that a wide variety of functional domains are enhanced by sleep, including visual (Karni et al. 1994), auditory (Eisner and McQueen 2006) and motor systems (Plihal and Born 1997; Walker et al. 2002) as well as inspiring insight into a hidden rule (Smith 1995, Yordanova et al. 2008). In contrast, research in children has not found evidence of sleep-dependent memory consolidation on implicit tasks such as mirror tracing (Prehn-Kristensen et al. 2009), serial reaction time involving motor memory (Fischer et al. 2007) and a finger tapping task for learning a motor sequence (Wilhelm et al. 2008) which, in adults, consistently improves following sleep (Walker et al. 2002). Conversely, Dimitriou et al. (2013) found a significant sleep-dependent improvement on the finger tapping task in 15 children aged 6 to 12 years.

More complex tasks involving implicit cognitive learning also improve following sleep in adults; for example, the Tower of Hanoi task, a mathematical puzzle that can easily be solved following discovery of the hidden rule. To our knowledge this task has not hitherto been used to assess sleep-dependent memory consolidation in children. Off-line improvement on the Tower of Hanoi has been linked to increased REMs and REM density on the post-learning night and can be disrupted by total sleep deprivation or REM deprivation after learning (Smith 1995; Smith et al. 2004). Conversely, Yordanova et al. (2008) found that, in adults, the extraction of a hidden arithmetic rule to a level where it could be explicitly stated was promoted by SWS rather than REM.

Wilhelm et al. (2012a) reported sleep-dependent improvement on an implicit sequence learning task only when children (aged 4 to 6 years) had been previously trained to an intermediate level of performance. Low-performing children did not significantly improve on the task following sleep. The authors suggest that implicit and explicit task components compete for off-line consolidation. Sleep preferentially enhances explicit aspects, thus

implicit memory representations may only be consolidated off-line if they are strong enough to avoid interference from competing gains in explicit knowledge (for a review see Wilhelm et al. 2012b). Using the same task, children (aged 8 to 12 years) and adults showed implicit learning gains over the course of training (reduced reaction times in response to a learnt sequence), but only following sleep were they able to explicitly freely recall the sequence that they had learnt. This effect was most notable in children and seemed to be related to their high level of SWS (Wilhelm et al. 2013).

The scarcity of studies in children, coupled with the importance of sleep for learning and therefore educational performance, demands further research as findings in adults cannot be generalized to children. Objective examination of the effects of sleep and the mechanisms underlying learning and memory will allow parents, teachers and clinicians to be better informed and to develop strategies to optimize sleep and learning.

The present study uses actigraphy to measure sleep patterns and assess sleep-dependent memory consolidation on an implicit and an explicit learning task in school-aged children.

Based on previous findings, we predict that i) children will show improvements on the explicit task but not the implicit task following the sleep period; ii) performance improvements will be linked to increased sleep quality and/or duration; iii) chronological age-related effects will be evident on sleep patterns and task performance.

Method

Participants

Thirty three typically developing children (17 male) aged 6 to 12 years (mean: 9.31 ± 1.52) were recruited through three local primary schools in London, UK. Children had normal, or corrected to normal, hearing and vision and no known sleep problems. Mental age (based on Raven's Coloured Progressive Matrices) was concordant with their chronological age. Ethical approval was granted by The Institute of Education, University of London Ethics

Committee. All parents provided written informed consent and children were asked for their consent verbally prior to taking part.

Actigraphy

Sleep patterns were measured using actigraphy (movement monitoring), a reliable and valid method for assessing sleep and wake, which shows more than 80% agreement with detection of sleep by overnight polysomnographic studies. It can be used to measure activity levels in a naturalistic setting over a prolonged period of time, but is unable to determine sleep stages (Acebo et al., 1999).

Each child was requested to wear an Actiwatch Mini (CamNTEch, Cambridge, UK) on the non-dominant wrist, continuously for one week (Acebo et al. 1999). Data were analyzed in one-minute epochs using Sleep Analysis 7 (CamNTEch, Cambridge, UK). In addition, parents completed a sleep log to support analyses of actigraphy data.

Actigraphy variables of interest include those relating to sleep duration: bed time, assumed sleep (total time from falling asleep to waking up) and actual sleep time (assumed sleep minus any periods of wake); as well as sleep quality: sleep efficiency (percentage of time spent asleep from sleep onset to wake up), sleep latency (time from parentally-reported lights out to sleep onset), number of night wakings, and fragmentation (an indication of restlessness where a higher figure indicates increased restlessness).

Experimental tasks

During the week in which children's sleep was examined, they also completed two tasks assessing sleep-dependent memory consolidation: a newly developed, explicit task (Animal Names) and an implicit task (Tower of Hanoi). In order to control for possible circadian effects on learning, half of the children were trained on the tasks in the morning (Wake-sleep group), the other half trained in the evening (Sleep-wake group). They were then tested twice at approximately 12 and 24 hours post-training following intervals of wake

and sleep (Figure 1). Evening tests were conducted at the child's home, with times ranging from 18:00 to 20:30 depending on bedtime. Morning sessions usually occurred at the child's school. The effect of different contexts on learning on retrieval was minimised by always testing children individually in a quiet room, seated at a table and without other distractions. To avoid interference that may occur by wake during the sleep retention interval, children were tested as soon as they arrived at school or just after registration, usually around 9am. They were also requested not to partake in any cognitively demanding activities, such as school work or music practice, between the evening and following morning test sessions.

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Figure 1 about here

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The average time interval was 10:25 hours between morning and evening testing (range: 9:00 to 11:45 hours), and 13:14 hours between evening and morning testing (range: 12:05 to 15:00 hours). These time differences were unavoidable due to variations in children's bedtimes and school start times and the need to minimize disruption to normal routines.

Animal Names task. The current task incorporates elements of both word pair and novel non-word learning and was specifically designed to improve on the frequently-used word pairs task for testing children. Instructions were simpler, the concept could be easily understood, and its nature made it more interesting and engaging. Ten farm and domestic animals were chosen as an anchor for learning non-words as realistic-sounding animal names. These were Basco the Cat, Razz the Chicken, Artoo the Cow, Kobi the Dog, Spyro the goat, Orin the Horse, Galba the Mouse, Jaala the Pig, Dax the Rabbit and Eagus the Sheep (see Figure 2 for example images).

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Figure 2 about here

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Attractive, coloured images of the animals were printed and laminated onto A6-sized white flashcards. After ensuring that children recognized all animals, they were told that they were going to be taught their personal names. Stimuli were presented one by one in a random order at a viewing distance of approximately 45cm. Children were told each animal's name, for example, the experimenter would say "This is Dax the Rabbit". They were asked to repeat each name to ensure they had heard and could pronounce it correctly. The experimenter then repeated, "Yes, Dax the Rabbit" or "No, Dax. Can you say it?" if the child said the name incorrectly, and allowed approximately three seconds pause before continuing to the next animal. Once complete for all cards, they were shuffled to randomize the order and minimize primacy and recency effects. Children were shown the cards a second time, asked if they could remember each name and were given feedback with the correct name. This was repeated a further three times so that children were shown the cards five times in total and all had the same level of exposure to the novel words. This took around 15 minutes to complete. Children then completed the Tower of Hanoi task (described in the next section) to remove any chance of immediate rehearsal. Finally, they were tested on the animal names and were given no feedback. The scoring system was two points for a correct answer and one point for an almost correct answer if one phoneme was incorrect, for example "Tobi the Dog" (instead of Kobi). Points were not awarded where children gave a correct name for the wrong animal. Following retention intervals of wake and sleep, children were shown the cards in a random order and asked if they could remember each name. They were not given feedback.

Tower of Hanoi. The Tower of Hanoi is a mathematical puzzle with the objective of moving all of a pile of stackable disks of different diameters from one peg to a third peg in as

few moves as possible ($2^n - 1$ where n is the number of disks). Only one disk may be moved at a time and no disk may be placed on top of a smaller disk. Performance depends upon discovery of the hidden rule to aid task completion, as well as executive ability to plan moves.

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Figure 3 about here

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The experimenter explained the rules to the child, and ensured they understood. They were told that they should plan their moves carefully and try to complete the puzzle in as few moves as possible using five disks (= 31 moves).

Children were trained by completing the task five times. After retention intervals of wake and sleep they completed the task twice during each session. The rules were reiterated at the start of each session. This procedure was designed to allow children to become familiar with the task during the first session and then not have too much practice in the following sessions so that improvement could not be due to rehearsal.

If a child lifted the disk from a peg and placed it back on the same peg, it was counted as one move. If they touched or lifted a disk but it remained on the peg, it was not counted as a move. Mean score of the final two trials of the training session and mean score of each test session were calculated.

Results

Data were analyzed using SPSS 18 and screened prior to analysis for outliers using Cooks distances. Children with outlying scores at any session were removed from the analysis of the respective task. Removal of outliers did not affect the significance of findings. Data were removed from the Animal Names task for one child at ceiling level and two children with outlying scores. On the Tower of Hanoi five children with outlying scores were

removed. Final participant details are presented in Table 1. T-tests and Chi-squared respectively showed no significant age or sex differences between the Sleep-wake and Wake-sleep groups for either task (all p values $>.05$).

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Table 1 about here

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Sleep Characteristics

Initially, actigraphy parameters were investigated for the whole sample and confirmed that no children had abnormal sleep patterns (Table 2). Analysis of Variance (ANOVA) showed that there was a significant difference between the Sleep-wake and Wake-sleep groups on only one variable: sleep latency (Sleep-wake: $22:44 \pm 09:18$, Wake-sleep: $28:46 \pm 06:21$, $F(1,27)=4.30$, $p<.05$, $\eta_p^2=.14$), indicating that the groups were well matched. Sleep latency depends on the accuracy of recording of parental diaries and is the least reliable actigraphy measure.

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Table 2 about here

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Animal Names Task

Animal Names data were analyzed in a 3x2 ANOVA with the within-subjects factor of session (Train, Test 1, Test 2) and between-subjects group (Sleep-wake, Wake-sleep). This showed a significant main effect of session (Wilks' Lambda=.59, $F(2, 27)=9.40$, $p<.001$,

$\eta_p^2=.41$). The interaction between group and session approached significance (Wilks' Lambda =.83, $F(2,27)=2.83$, $p=.08$, $\eta_p^2=.17$).

One-way repeated measures ANOVAs were then conducted to compare scores on the Animal Names task between the three sessions for each group (Table 3). There was a significant effect of session for both groups (Sleep-wake: $F(1.28,15.34)=24.39$, $p<.001$, $\eta_p^2=.82$; Wake-sleep: $F(2,15)=5.35$, $p<.05$, $\eta_p^2=.42$). Mauchly's test indicated that sphericity could not be assumed for the Sleep-wake group ($X^2(2)=9.14$, $p<.05$); therefore degrees of freedom were adjusted according to Greenhouse-Geisser estimates of sphericity ($\epsilon=.64$).

Post-hoc tests using the Bonferroni correction indicated that both the Sleep-wake ($p<.001$) and Wake-sleep ($p=.05$) groups significantly improved on the Animal names task following sleep, but not wake. The mean improvement for both groups together was 14.03% following sleep and 2.18% following wake. A repeated measures ANOVA comparing these scores indicated significantly greater improvement following sleep than wake ($F(1,29)=5.17$, $p=.03$, $\eta_p^2=.59$).

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Table 3 about here

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Tower of Hanoi Task

Tower of Hanoi data were analyzed in a 3x2 ANOVA with the within-subjects factor of session (Train, Test 1, Test 2) and between-subjects group (Sleep-wake, Wake-sleep). There was a significant main effect of session (Wilks' Lambda =.40, $F(2,25)=18.91$, $p<.001$, $\eta_p^2=.60$) and interaction between group and session (Wilks' Lambda =.54, $F(2,25)=10.48$, $p<.001$, $\eta_p^2=.46$). A one-way repeated measures ANOVA was then conducted for each group to compare scores between the three sessions (Table 4). There was a significant effect of

session for both groups (Sleep-wake: $F(2,13)=7.62, p<.01, \eta_p^2=.54$; Wake-sleep: $F(2,11)=18.10, p<.001, \eta_p^2=.77$).

Post-hoc tests using the Bonferroni correction indicated that both the Sleep-wake ($p=.01$) and Wake-sleep ($p<.001$) groups significantly improved on the Tower of Hanoi task following sleep, but not wake. Note that a lower score here indicates better performance, since the aim is to complete the task in as few moves as possible. The mean change in score for both groups was -25.01% following sleep and +2.70% following wake. A repeated measures ANOVA comparing these scores indicated significantly greater improvement following sleep than wake ($F(1,27)=11.49, p=.002, \eta_p^2=.90$).

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Table 4 about here

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Age effects

Sleep parameters and task scores were correlated with chronological age using Pearson's product-moment correlation coefficients. There was a significant positive correlation between age and bed time with older children going to bed later ($r(32)=.57, p<.001$), and a general non-significant trend for improved sleep quality with increasing age.

Older children remembered significantly more animal names than younger children ($r(30)=.50, p<.01$). In contrast, age was not related to performance on the Tower of Hanoi task ($r(28)=.004, p>.05$).

Sleep effects

Partial correlations were used to control for age whilst investigating children's sleep and performance on the tasks. It was expected that children with increased mean sleep quality and/or duration would perform better on the tasks initially and that sleep quality and/or duration on the night of the test would be related to greater improvement on the tasks;

however, no statistically significant partial correlations regarding sleep quality were found (all p values $>.05$).

Discussion

The present study successfully implemented a novel explicit and an implicit learning task in a group of 33 children to show that children's learning on both tasks was preferentially enhanced by sleep compared to wake.

The newly developed Animal Names task proved to be sensitive across the ages tested as only one child performed at ceiling and no children were at floor level. Similarly to word pair studies in children (e.g., Wilhelm et al. 2008), memory was significantly enhanced by sleep compared to wake, regardless of whether initial training took place in the morning or evening. This supports our hypothesis and confirms reliability of our task. We therefore suggest that the memory traces for the Animal Names were actively reinforced during sleep,

The implicit learning task used in the present study also yielded an improvement in children's performance following the sleep period. This is in contrast with most other published data for implicit tasks in children (Fischer et al. 2007; Wilhelm et al. 2008), although others have reported similar findings more recently (Wilhelm et al. 2012a; Dimitriou et al. 2013).

There are a number of possible explanations for our novel findings. The Tower of Hanoi differs from some other implicit tasks as it also involves a cognitive aspect to discover the hidden rule. Whilst Smith et al. (2004) found that learning on this task in adults was disrupted by REM deprivation, Yordanova et al. (2008) and Wilhelm et al. (2013) suggest that hidden rule extraction is related to SWS, not REM, and thus lends itself to offline consolidation in children due to their high levels of SWS. Conversely, tasks that rely purely on implicit skill gains, such as mirror tracing or finger tapping, may not be sleep-dependent in children. This suggests that sleep-dependent memory consolidation is affected by specific

features of the learning task. Alternatively, as Fischer et al. (2007) and Wilhelm et al. (2012b) suggest, childhood sleep preferentially enhances explicit rather than implicit memory. Whilst in adults sleep aids the discovery of the hidden rule, it is possible that children learn the task through different means, for example, explicitly remembering sequences that have ‘worked’ on previous trials. Future studies could attempt to investigate tasks that involve only one aspect of memory to enable better understanding of how different skills are enhanced by sleep. Ideally, learning and retrieval contexts would also be controlled to avoid this possible confounding factor. In addition, targeting older children could ascertain when purely implicit sleep-dependent memory consolidation is acquired. In the real world, learning rarely relies only on a single type of memory, so learning techniques could be maximized to make use of off-line memory consolidation.

It was expected that performance on the learning tasks would be related to sleep quality and/or duration; however, this was not the case, probably because actigraphy is not sensitive enough to provide information about stage-dependent correlates to learning that are possible with PSG. Children were selected to exclude those with known sleep problems and their sleep was in the normal range. Thus, it is probable that all were engaging in adequate sleep to perform well on the tasks. Future studies could examine this dimension by comparing sleep-dependent memory consolidation and baseline performance in good and poor sleepers, or experimental manipulation of sleep quality and/or duration.

One could argue that the overnight gains seen in our study were simply due to children being tested in the morning when they were less tired than in the evening, in which case one would also expect to see a decline in performance over the daytime, which is not reflected in our data. In addition, sleep did not simply protect new memories from interference or forgetting. Had this been the case, we would expect performance to have simply remained

stable overnight. On the contrary, children's performance improved following sleep, suggesting an active role of sleep in the consolidation of memory.

We expected to find more developmental effects in sleep patterns and performance on the tasks. Although older children tended to go to bed later than younger children, there were no other significant age-related effects in their sleep quality or duration, perhaps due to the relatively small age range examined here. The finding of an age-related association with performance on the Animal Names task but not on the Tower of Hanoi is interesting. It may reflect the greater pre-existing knowledge base in older children on which to anchor the newly learned Animal Names, whilst the Tower of Hanoi was a novel task for all children so none could rely on pre-existing knowledge.

The current study supports the notion that sleep is necessary for enhanced memory consolidation in children and reinforces the importance of sleep for children to maximize learning potential. Designing and implementing effective programs to optimize learning need to factor in children's night-time sleep, for example by testing children on their homework the following morning to reinforce any sleep-related gains. Although implicit learning in children has not always been found to be enhanced by sleep, future research could investigate the precise aspects of implicit tasks that benefit from off-line consolidation. There is now no doubt that there is a complex interplay between cognition and sleep. Hence educationists, researchers and clinicians must understand the importance of sleep and promote a culture to children and parents that values sleep as an aid to learning and development.

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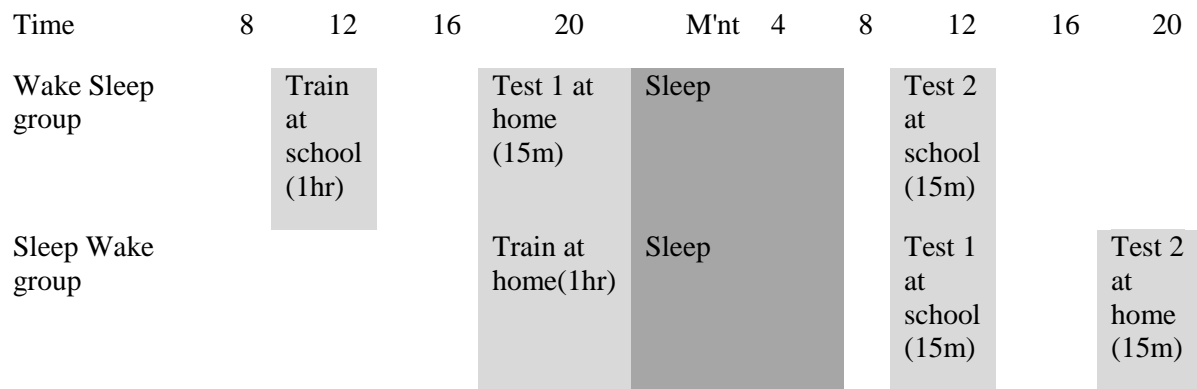


Figure 1. Testing schedule for Wake-sleep and Sleep-wake groups.

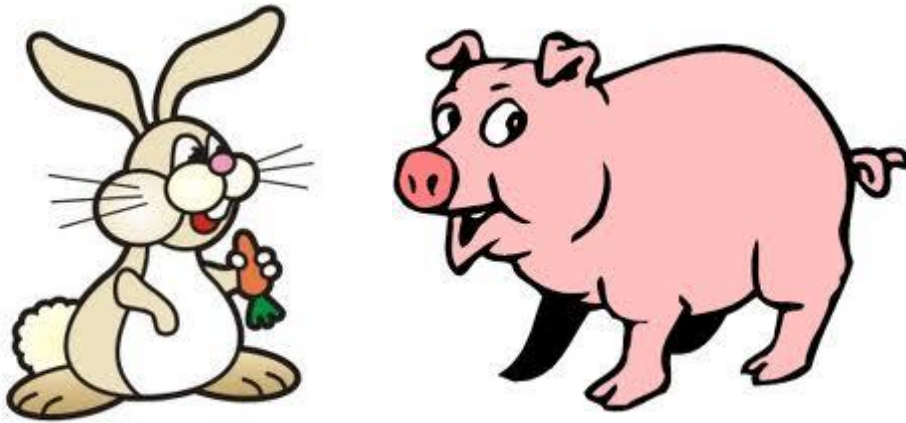


Figure 2. Example of Animal Names flashcard images: Dax the Rabbit and Jaala the Pig.



Figure 3. The Tower of Hanoi Task.

Table 1

Participant details for Animal Names and Tower of Hanoi tasks

Task	Group	N	Male / female	Mean age (SD)	Age range
Animal Names	Sleep-wake	13	6 / 7	9.61 (1.51)	7.15 - 11.98
	Wake-sleep	17	10 / 7	9.19 (1.65)	6.19 - 12.02
	Total	30	16 / 14	9.37 (1.58)	6.16 - 12.02
Tower of Hanoi	Sleep-wake	15	6 / 9	9.50 (1.43)	7.15 - 11.98
	Wake-sleep	13	6 / 7	9.06 (1.40)	6.28 - 11.09
	Total	28	12 / 16	9.30 (1.41)	6.28 - 11.98

Table 2

Sleep parameters from actigraphy

Sleep parameter	Mean	SD	Minimum	Maximum
Bed time (hh:mm)	21:21	00:34	20:22	22:32
Assumed sleep time (hh:mm)	09:32	00:32	08:23	10:12
Actual sleep time (hh:mm)	08:23	00:33	07:09	09:08
Sleep efficiency (%)	88.18	3.30	81.29	93.68
Sleep latency (mm:ss)	26:04	08:15	12:30	42:51
Number of night wakings	31.26	7.89	16.86	45.14
Fragmentation	28.44	7.62	17.13	44.47

Table 3

Results for Repeated Measures ANOVA on Animal Names task

Group	Session	Mean score	SD	% change in score	F	<i>p</i>	η_p^2
Sleep-wake	Train (pm)	10.77	3.59				
	Test 1 (am)	12.69	3.17	17.83	44.64	<.001	.79
	Test 2 (pm)	12.69	3.35	0	.00	1.00	.00
Wake-sleep	Train (am)	10.65	4.40				
	Test 1 (pm)	11.06	3.40	3.85	.38	.55	.02
	Test 2 (am)	12.29	3.67	11.12	7.03	.02	.31

Table 4

Results for Repeated Measures ANOVA on Tower of Hanoi task

Group	Session	Mean moves	SD	% change in score	F	<i>p</i>	η_p^2
Sleep-wake	Train (pm)	55.73	11.21				
	Test 1 (am)	45.53	7.97	-18.30	12.47	.003	.47
	Test 2 (pm)	44.53	6.27	-2.20	.15	.70	.01
Wake-sleep	Train (am)	63.08	16.01				
	Test 1 (pm)	68.35	17.85	8.35	.60	.45	.05
	Test 2 (am)	45.96	8.10	-32.76	32.49	<.001	.73